

1. An antibody or a functional fragment thereof, binding to TRAIL-R1 and/or TRAIL-R2.
2. The antibody or the functional fragment thereof of claim 1, having at least one property selected from the following (a) to (c) of:
  - (a) having activity to induce apoptosis in carcinoma cells expressing TRAIL-R1 and/or TRAIL-R2;
  - (b) not having effect on normal human cells expressing TRAIL-R1 and/or TRAIL-R2; and
  - (c) not inducing human hepatocyte toxicity.
3. An antibody or a functional fragment thereof, having all the following properties (a) to (c) of:
  - (a) having activity to induce apoptosis in carcinoma cells expressing TRAIL-R1 and/or TRAIL-R2;
  - (b) not having effect on normal human cells expressing TRAIL-R1 and/or TRAIL-R2; and
  - (c) not inducing human hepatocyte toxicity.
4. The antibody or the functional fragment thereof of claim 2 or 3, which binds to TRAIL-R2, but does not bind to TRAIL-R1.
5. The antibody or the functional fragment thereof of claim 2 or 3, which binds to both TRAIL-R2 and TRAIL-R1.
6. The antibody or the functional fragment thereof of any one of claims 1 to 5, which is a monoclonal antibody produced by a mouse-mouse hybridoma.
7. The antibody or the functional fragment thereof of any one of claims 1 to 6, which is a human antibody.
8. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 0.01 µg/ml or more for human hepatocytes when the number of cells is  $7.5 \times 10^4$  and the reaction time is 24 hours.

having an LD50 value of 0.1 µg/ml or more for human hepatocytes when the number of cells is  $7.5 \times 10^4$  and the reaction time is 24 hours.

10. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 2 to 10 µg/ml for human hepatocytes when the number of cells is  $7.5 \times 10^4$  and the reaction time is 24 hours.

11. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 10 µg/ml or more for human hepatocytes when the number of cells is  $7.5 \times 10^4$  and the reaction time is 24 hours.

12. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 10 to 100 µg/ml for human hepatocytes when the number of cells is  $7.5 \times 10^4$  and the reaction time is 24 hours.

13. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 100 µg/ml or more for human hepatocytes when the number of cells is  $7.5 \times 10^4$  and the reaction time is 24 hours.

14. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 100 µg/ml or less for carcinoma cells when the number of cells is  $2.5 \times 10^3$  and the reaction time is 48 hours.

15. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 10 µg/ml or less for carcinoma cells when the number of cells is  $2.5 \times 10^3$  and the reaction time is 48 hours.

16. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 0.7 µg/ml or less for carcinoma cells when the number of cells is  $2.5 \times 10^3$  and the reaction time is 48 hours.

17. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 0.02 to 0.11 µg/ml for carcinoma cells when the number of cells is  $2.5 \times 10^3$  and the reaction time is 48 hours.

18. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 0.02 µg/ml or less for carcinoma cells when the

19. The antibody or the functional fragment thereof of any one of claims 1 to 7, having an LD50 value of 2 to 100  $\mu\text{g/ml}$  for human hepatocytes when the number of cells is  $7.5 \times 10^4$  and the reaction time is 24 hours, and having an LD50 value of 0.02 to 0.11  $\mu\text{g/ml}$  for carcinoma cells when the number of cells is  $2.5 \times 10^3$  and the reaction time is 48 hours.

20. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is  $7.5 \times 10^4$  and the reaction time is 24 hours is 2 times or more greater than the LD50 value for carcinoma cells when the number of cells is  $2.5 \times 10^3$  and the reaction time is 48 hours.

21. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is  $7.5 \times 10^4$  and the reaction time is 24 hours is 10 times or more greater than the LD50 value for carcinoma cells when the number of cells is  $2.5 \times 10^3$  and the reaction time is 48 hours.

22. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is  $7.5 \times 10^4$  and the reaction time is 24 hours is 50 times or more greater than the LD50 value for carcinoma cells when the number of cells is  $2.5 \times 10^3$  and the reaction time is 48 hours.

23. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is  $7.5 \times 10^4$  and the reaction time is 24 hours is 50 to 100 times greater than the LD50 value for carcinoma cells when the number of cells is  $2.5 \times 10^3$  and the reaction time is 48 hours.

24. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is  $7.5 \times 10^4$  and the reaction time is 24 hours is 100 times or more greater than the

reaction time is 48 hours.

25. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is  $7.5 \times 10^4$  and the reaction time is 24 hours is 100 to 1000 times greater than the LD50 value for carcinoma cells when the number of cells is  $2.5 \times 10^3$  and the reaction time is 48 hours.

26. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is  $7.5 \times 10^4$  and the reaction time is 24 hours is 250 to 1000 times greater than the LD50 value for carcinoma cells when the number of cells is  $2.5 \times 10^3$  and the reaction time is 48 hours.

27. The antibody or the functional fragment thereof of any one of claims 1 to 7, wherein the LD50 value for human hepatocytes when the number of cells is  $7.5 \times 10^4$  and the reaction time is 24 hours is 1000 times or more greater than the LD50 value for carcinoma cells when the number of cells is  $2.5 \times 10^3$  and the reaction time is 48 hours.

28. The antibody or the functional fragment thereof of any one of claims 8 to 27, wherein a reaction volume is between 110 and 120  $\mu$ l.

29. The antibody or the functional fragment thereof of any one of claims 2, 3 and 14 to 28, wherein the carcinoma cells are Colo205 cells.

30. The antibody or the functional fragment thereof of claim 2 or 3, wherein the carcinoma cells are Colo205 cells, U251 cells or Jurkat cells.

31. The antibody or the functional fragment thereof of any one of claims 1 to 30, which can suppress tumor growth or regress tumors.

32. The antibody or the functional fragment thereof of claim 31, wherein the tumor is at least one tumor selected from the group consisting of colon cancer, colorectal cancer, lung cancer, breast cancer, brain tumor, malignant melanoma, renal cell carcinoma, bladder cancer, leukemia, lymphomas, T cell lymphomas,

carcinoma, ovarian cancer, esophageal cancer, liver cancer, head and neck squamous cell carcinoma, cutaneous cancer, urinary tract carcinoma, prostate cancer, choriocarcinoma, pharyngeal cancer, laryngeal cancer, thecomatosis, androblastoma, endometrium hyperplasia, endometriosis, embryoma, fibrosarcoma, Kaposi's sarcoma, hemangioma, cavernous hemangioma, angioblastoma, retinoblastoma, astrocytoma, neurofibroma, oligodendroglioma, medulloblastoma, ganglioneuroblastoma, glioma, rhabdomyosarcoma, hamartoblastoma, osteogenic sarcoma, leiomyosarcoma, thyroid sarcoma, Wilms tumor and the like.

33. The antibody or the functional fragment thereof of claim 31, wherein the tumor is derived from Colo205 cells transplanted in a nude mouse.

34. The antibody or the functional fragment thereof of any one of claims 31 to 33, wherein a period during which tumor growth can be suppressed or tumor regression can be achieved is at least 9 days.

35. The antibody or the functional fragment thereof of any one of claims 31 to 34, wherein the dose of the antibody or the functional fragment thereof is 100  $\mu\text{g}/\text{body}$  or 5 mg/kg.

36. The antibody or the functional fragment thereof of any one of claims 31 to 34, wherein the dose of the antibody or the functional fragment thereof is 20  $\mu\text{g}/\text{body}$  or 1 mg/kg.

37. The antibody or the functional fragment thereof of any one of claims 31 to 34, wherein the dose of the antibody or the functional fragment thereof is 4  $\mu\text{g}/\text{body}$  or 200  $\mu\text{g}/\text{kg}$ .

38. The antibody or the functional fragment thereof of any one of claims 31 to 34, wherein the dose of the antibody or the functional fragment thereof is 1  $\mu\text{g}/\text{body}$  or 50  $\mu\text{g}/\text{kg}$ .

39. The antibody or the functional fragment thereof of any one of claims 1 to 38, which is an immunoglobulin G antibody.

of 14% or more tumor reduction by 4 days after the initial administration, when administered at a concentration of 20 µg/mouse to a 4- to 6-week-old tumor-bearing mouse having a 100 mm<sup>3</sup> tumor.

41. The antibody or the functional fragment thereof of claim 40, which can maintain an average of 14% or more tumor reduction for at least 7 days.

42. The antibody or the functional fragment thereof of claim 40, which can induce an average of 65% or more tumor reduction by 4 days after the initial administration, when administered at a concentration of 20 µg/mouse to a 4- to 6-week-old tumor-bearing mouse having a 100 mm<sup>3</sup> tumor.

43. The antibody or the functional fragment thereof of claim 40, which can induce an average of 80% or more tumor reduction by 7 days after the initial administration, when administered at a concentration of 20 µg/mouse to a 4- to 6-week-old tumor-bearing mouse having a 100 mm<sup>3</sup> tumor.

44. The antibody or the functional fragment thereof of claim 43, which can maintain an average of 80% or more tumor reduction for at least 4 days.

45. The antibody or the functional fragment thereof of claim 40, which can induce an average of 45% or more tumor reduction by 3 days after the initial administration, when administered at a concentration of 25 µg/mouse to a 12-week-old tumor-bearing mouse having a 100 mm<sup>3</sup> tumor.

46. The antibody or the functional fragment thereof of claim 45, which can induce an average of 65% or more tumor reduction by 5 days after the initial administration, when administered at a concentration of 25 µg/mouse to a 12-week-old tumor-bearing mouse having a 100 mm<sup>3</sup> tumor.

47. The antibody or the functional fragment thereof of claim 46, which can maintain an average of 65% or more tumor reduction for at least 27 days.

48. The antibody or the functional fragment thereof of claim 40, which can induce an average of 39% or more tumor reduction by 4 days after the initial administration, when administered at a concentration of 20 µg/mouse to a 4- to

49. The antibody or the functional fragment thereof of claim 48, which can maintain an average of 39% or more tumor reduction for at least 14 days.

50. The antibody or the functional fragment thereof of claim 40, which is a 0304 antibody.

51. The antibody or the functional fragment thereof of claim 40, which is an E-11-13 antibody.

52. An antibody or a functional fragment thereof binding to TRAIL-R1 and/or TRAIL-R2, which is produced by a hybridoma E-11-13, H-48-2, L-30-10, N-18-12, W-40-5, X-14-4, X-51-12, F-4-8, G-3-10, 0304 or KMTR1.

53. An antibody or a functional fragment thereof binding to TRAIL-R1 and/or TRAIL-R2, which is produced by a hybridoma H-48-2 with the accession number of FERM BP-7599, a hybridoma E-11-13 with the accession number of FERM BP-7698 or FERM BP-7770, a hybridoma F-4-8 with the accession number of FERM BP-7699 or FERM BP-7768, a hybridoma L-30-10 with the accession number of FERM BP-7700 or FERM BP-7769, a hybridoma 0304 with the accession number of FERM BP-8037, or a hybridoma KMTR1 with the accession number of FERM BP-8038.

54. An antibody or a functional fragment thereof, having amino acid sequences of the mature portions of a heavy chain variable region and a light chain variable region of the antibody produced by a hybridoma E-11-13, which are respectively represented by SEQ ID NOS: 17 and 19; a heavy chain variable region and a light chain variable region of the antibody produced by a hybridoma L-30-10, which are respectively represented by SEQ ID NOS: 21 and 23; a heavy chain variable region and a light chain variable region of the antibody produced by a hybridoma H-48-2, which are respectively represented by SEQ ID NOS: 25 and 27; a heavy chain variable region and a light chain variable region of the antibody produced by a hybridoma 0304, which are respectively represented by SEQ ID NOS: 29 and 31; or a heavy chain variable region and a light chain

respectively represented by SEQ ID NOS: 33 and 35.

55. An antibody or a functional fragment thereof, having amino acid sequences of the mature portions of a heavy chain variable region and a light chain variable region that are encoded by nucleic acid sequences isolated from a hybridoma E-11-13, which are respectively represented by SEQ ID NOS: 16 and 18; a heavy chain variable region and a light chain variable region that are encoded by nucleic acid sequences isolated from a hybridoma L-30-10, which are respectively represented by SEQ ID NOS: 20 and 22; a heavy chain variable region and a light chain variable region that are encoded by nucleic acid sequences isolated from a hybridoma H-48-2, which are respectively represented by SEQ ID NOS: 24 and 26; a heavy chain variable region and a light chain variable region that are encoded by nucleic acid sequences isolated from a hybridoma 0304, which are respectively represented by SEQ ID NOS: 28 and 30; or a heavy chain variable region and a light chain variable region that are encoded by nucleic acid sequences isolated from a hybridoma KMTR1, which are respectively represented by SEQ ID NOS: 32 and 34.

56. A hybridoma producing monoclonal antibodies that bind to TRAIL-R2, which is selected from the group consisting of E-11-13, H-48-2, L-30-10, N-18-12, W-40-5, X-14-4, X-51-12, F-4-8, G-3-10, 0304 and KMTR1.

57. A hybridoma producing monoclonal antibodies that bind to TRAIL-R2, which is selected from a hybridoma H-48-2 with the accession number of FERM BP-7599, a hybridoma E-11-13 with the accession number of FERM BP-7698 or FERM BP-7770, a hybridoma F-4-8 with the accession number of FERM BP-7699 or FERM BP-7768, a hybridoma L-30-10 with the accession number of FERM BP-7700 or FERM BP-7769, a hybridoma 0304 with the accession number of FERM BP-8037 and a hybridoma KMTR1 with the accession number of FERM BP-8038.

58. A method for producing anti-TRAIL-R2 monoclonal antibodies, comprising



to TRAIL-R2 from the obtained culture product.

59. A method for producing anti-TRAIL-R2 monoclonal antibodies, comprising isolating a gene encoding an anti-TRAIL-R2 monoclonal antibody from the hybridoma of claim 56 or 57, constructing an expression vector having the gene, introducing the expression vector into a host to express the monoclonal antibody, and collecting anti-TRAIL-R2 monoclonal antibodies from the obtained host, or the culture supernatant or the secretion of the host.

60. The production method of claim 59, wherein the host is any host selected from the group consisting of *Escherichia coli*, yeast cells, insect cells, mammalian cells and plant cells, and mammals.

61. A prophylactic or therapeutic agent against tumors, comprising as an active ingredient the antibody or the functional fragment thereof of any one of claims 1 to 55.

62. The prophylactic or therapeutic agent of claim 61, wherein the tumor is any one tumor selected from the group consisting of colon cancer, colorectal cancer, lung cancer, breast cancer, brain tumor, malignant melanoma, renal cell carcinoma, bladder cancer, leukemia, lymphomas, T cell lymphomas, multiple myeloma, gastric cancer, pancreas cancer, cervical cancer, endometrial carcinoma, ovarian cancer, esophageal cancer, liver cancer, head and neck squamous cell carcinoma, cutaneous cancer, urinary tract carcinoma, prostate cancer, choriocarcinoma, pharyngeal cancer, laryngeal cancer, thecomatosis, androblastoma, endometrium hyperplasy, endometriosis, cmbryoma, fibrosarcoma, Kaposi's sarcoma, hemangioma, cavernous hemangioma, angioblastoma, retinoblastoma, astrocytoma, neurofibroma, oligodendroglioma, medulloblastoma, ganglioneuroblastoma, glioma, rhabdomyosarcoma, hamartoblastoma, osteogenic sarcoma, leiomyosarcoma, thyroid sarcoma, Wilms tumor and the like.